First evaluation of a new ^{19}F-derivative of sulfonylurea ligands for the determination of the beta-cell status *in vivo*

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Intoduction:
Diabetes mellitus comprises a heterogeneous group of disorders characterized by high blood glucose levels. Two major types of diabetes mellitus have been defined: type 1 (insulin-dependent diabetes mellitus, IDDM), and type 2 (non-insulin-dependent diabetes mellitus, NIDDM). Although hyperglycemia is the common denominator of both IDDM and NIDDM, the etiology and pathophysiology of these syndromes are distinct. IDDM is a chronic autoimmune disease characterized by the selective destruction of insulin-producing β-cells of the islets of Langerhans. When autoimmune destruction affects more than 90% of the β-cell mass, the resulting insulin deficiency culminates into development of overt hyperglycemia. In NIDDM, on the other hand, the pancreatic β-cells are initially intact, and the disease is associated with insulin resistance and loss of β-cell function, and eventual insulin-dependency [1].
Tolbutamide with a K_i of 25-55 µM and glibenclamide with a K_i of 0.7-7 nM are sulfonurea agents used to stimulate insulin secretion in type 2 diabetic patients [2]. We intend to determine the efficacy of these radiolabeled agents in visualizing and quantifying β-cell concentrations in the pancreas of normal non-human primates by PET.
The aim of this study was to evaluate the β-cell-specific positron emitting radiolabeled sulfonylurea derivative 2-[^{19}F]fluoroethyl-tolbutamide *in vitro*.

First of all, the ^{19}F-compounds of the described sulfonylurea derivatives were synthesized for testing their ability to stimulate insulin secretion from pig beta-cells. In the case of the tolbutamide derivative the synthesis started from 4-amino-benzensulfonamide, which was converted via diazotation and hydrolysis into 4-hydroxy-benzenesulfonamid. The hydroxy group was then alkylated with 2-fluoro-1-bromoethane under basic conditions and the remaining sulfonamide moiety was coupled with butylisocyanate to yield the desired 2-[^{19}F]fluoroethyl-tolbutamide (fig. 1).
The glibenclamide derivative was obtained by a multistep synthetic route. Starting from the di-sodium salt of 5-chloro-salicylic acid, 2-fluoro-1-bromoethan was added to yield the fluoroalkylated salicylic acid fluoroethylester which was then cleaved with 2 N NaOH solution to yield the fluoroalkylated salicylic acid derivative. The carboxyl group of this compound was activated via a mixed anhydride for further nucleophilic displacement with 4-(2-aminoethyl) benzenesulfonamide. The resulting compound was reacted with cyclohexylisocyanate and Cu(I) catalysis to give the desired glibenclamide derivative 2-[^{19}F]fluoroethyl-glibenclamide.

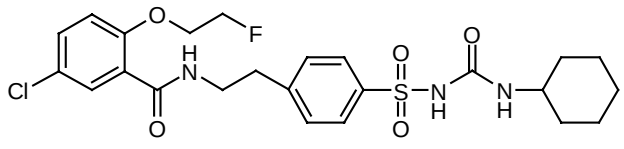

Fig. 1: 2-[^{19}F]fluoroethyl-glibenclamide

***In vitro* evaluation**:
For testing the *in vitro* function of the glibenclamide derivative 2-[^{19}F] fluoroethyl-glibenclamide a standardized batch stimulation was performed. Adult rat islets were isolated by collagenase digestion and purified by a density gradient. For each sample ten islets were used (equal in size and shape) for a culture-insert with a membrane of 3 µm pore size. First the basal insulin secretion was tested by culturing the islets with normo-glycemic culture-media (RPMI 1640 + D-glucose 100 mg/dl + 10% FCS + P/S) for 1 hour at 37°C. After the culture period the media were collected and stored at –20°C. The inserts with islets were transferred to normo-glycemic culture-media with several concentrations of the glibenclamide derivative and cultured for a second, stimulated period. As a positive control several inserts with islets were cultured with a hyperglycemic culture-media (RPMI 1640 D-glucose 300mg/dl + 10% FCS + P/S) only. For the negative control normo-glycemic culture-media (RPMI 1640 D-glucose 100mg/dl + 10% FCS + P/S) with a diluted-solution but without the glibenclamide derivative was used. The insulin content of each probe was quantified by a rat-insulin-ELISA. The stimulation effect (in %) was calculated as stimulated insulin secretion divided by basal insulin secretion * 100 (tab. 1)

Tab 1: Effects on insulin secretion of 2-[^{19}F]fluoroethyl-glibenclamide

	Concentration of 2-[^{19}F]fluoroethyl-glibenclamide [ng/ml]				
	0.025	0.25	2.5	Pos. control	Neg. control
Stimulation effect [%]	291.6	471.1	160.0	533.4	115.2
SD [+/- %]	62.2	101.5	9.8	80.6	13.1

Conclusion*:*
The non radioactive ^{19}F-standard compound 2-[^{19}F]fluoroethyl-glibenclamide was successfully evaluated in comparison to glibenclamide for the effect on insulin secretion. We could detect nearly the same stimulation effect of 0.25 ng/ml 2-[^{19}F]fluoroethyl-glibenclamide as in the hyperglycemic positive control. The evaluation of 2-[^{19}F]fluoroethyl-tolbutamide is under investigation.

[1] Ronner P et al. Diabetes, 42: 1760-72 (1993)
[2] Gribble F.M. et al. Diabetes , 47: 1412-8 (1998)